ESTIMATION OF SIALIC ACID CONTENT OF CERVICAL MUCUS AND ITS RELATIONSHIP TO FEMALE INFERTILITY

THESIS

FOR

MASTER OF SURGERY

(OBSTETRICS AND GYNAECOLOGY)

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JHANSI (U. P.)

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RAMA DEVI PRAJAPATI

C E R T I F I C A T E

This is to certify that the work on "Estimation of Sialic Acid content of cervical mucus and its relationship to female infertility" which is being submitted for M.S.(Obstetrics & Gynaecology) THESIS by RAMA DEVI PRAJAPATI, has been carried out under my supervision and guidance in the Department of Obstetrics & Gynaecology. The techniques embodied in the thesis were undertaken by the candidate herself and the observations recorded have been periodically checked by me.

She has fulfilled the necessary requirements of the stay in the department for the submission of the thesis.

Dated : 31.8.88

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This voyage was a success due to kind co-operation of the patients and their husbands who have formed the material of this study. I shall remain indebted to them for ever.

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Rama Devi Prajapati.

Dated : 31st August, 1968 (RAMA DEVI PRAJAPATI)

C O N T E N T S

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INTRODUCTION

I N T R O D U C T I O N

Barren marriage is an age old problem. With the advancement of civilisation, it has become more than an eternal medical problem which affects the welfare of society because reproduction is an essential aspect of marriage.

Exact incidence is 15% i.e. 1 in every 7 marriages. Positive universal interest in human fertility has become a part of many religions. Certainly, a condition, so prevalent, so undesirable and so threatening to society, through its affect on family life, warrants the close scrutiny, it is universally receiving.

"The association of a normal semen examination with a poor post-coital invasion, is a cause of sterility in 5-8% of barren women where other routine investigations of infertility do not show any abnormality as a cause".

Having settled, the presence of normal semen in the husband by previous examination of the ejaculate, it is necessary to determine whether or not the 'CERVIX' provides a physiologic medium for them.

Penetration of the cervical mucus by a large number of sperms is essential to fertility. This necessitates both the proper deposition of normal semen and a salutary state of the cervical canal. Cervix is a factor in sterility chiefly because of the pathological changes in the physical characteristics and the chemistry of its mucus.

The primary function of the preovulatory cervical secretion is to supply an easily penetrable, nutrient secretion for the spermatozoa that enter the cervical canal in their upward migration.

The term "hostility" employed in relation to cervical mucus in intended to imply that the normal appearing cervix may produce mucus that does not support and enhance spermatozoal passage.

The major changes in circulating blood levels of oestrogen and progesterone which occur during the menstrual cycle produce recognisable peripheral effects that can be used as signs of ovulation of which that on the cervical mucus is most important.

"The cervical factor must be looked for physically as well as biochemically because a cervix that appears to be normal on inspection with a speculum, is often abnormal biochemically and vice versa". Such a cervix though free from obvious abnormality, fails to do its physiologic function. It has "dysmucorrhoea". The fertile period of the menstrual cycle is governed to a large extent by oestrogen influences on cervical mucus secretion. Oestrogen along with progesterone exert significant controlling influences on fertility through their hormonal action on the cervix and particularly on the mucosa lining the crypts of the endocervical canal. This latter

action brings about cyclic changes in the nature of the mucus fluid secreted by the endocervix that are very important to sperm transport and survival in the female reproductive tract. Mid-cycle penetrable mucus is mainly the result of an oestrogen action and cervical mucus is the main factor involved in regulating sperm migration as the cervix is strategically situated at the entrance to the upper genital tract in which the reproductive processes of fertilisation, inplantation and development of pregnancy takes place.

When an opalescent, viscid, non-inflammatory cervical mucus, where in healthy spermatozoa may become enmeshed and devitalised, is present repeatedly during the ovulation phase of the menstrual cycle, a biochemical (endocrine) defect in the activity of the cervical glands may be assumed to be at the root of the trouble and even clear, elastic ovulatory cervical mucus may exhibit hostility. Such hostility may be merely an expression of insufficient oestrogen influence on the cervical glands.

In this presentation, one aspect of the pervasive philosophy that governs a logical approach to the problem of infertility i.e. the cervix and its mucus will be related to one knowledge of its structure. A hypothesis has been

postulated explaining the molecular biology of this mucus whose properties undergo unique changes during the menstrual cycle. The biochemical basis for these changing properties will be reviewed.

The sialic acid (a glycoprotein), an important constituent of the cervical mucus responsible for the rigidity and coherence of the mucin molecule has stimulated a voluminous literature and has been found applicable to many more conditions than the other tests of the cervical mucus. The clinical assessment of this cervical factor will be done in relation to its place in the investigation of the infertile couple.

REVIEW OF LITRATURE

REVIEW OF LITERATURE

Cervical mucus changes have been recognised as a means of identifying the likely time of ovulation. In contrast to the shift in basal body temperature, the oestrogen induced changes in the mucus and its secretion offer a prediction of the time of ovulation. As well, they give valuable information on potential fertility in regard to sperm transport and survival.

The physical and chemical properties of cervical mucus undergo cyclic changes in response to the changing pattern of ovarian steroids secretion (Zondek, 1957 ; Marcus & Marcus, 1965; Cohen, 1966; Elstein, 1970; Mac Donald & Lumley, 1970; Moghissi, 1972; Moghissi and Wallach, 1983). These include, appearance of cervix, mid-cycle mucorrhoea, spinnbarkeit, ferning, viscosity and changes in chemical composition of mucus.

The influence of oestrogen production in the ovary during the first part of the cycle, on the cervical mucus secretion was demonstrated by Seguy and Simmonet (1933) and others. Estrogen stimulates the production of profuse clear watery (due to more hygroscopic nature) , alkaline, a-cellular mucus with increased flow elasticity and ferning and thinner viscosity. Water content ranges from 96-98% and sodium chloride makes 90% of inorganic salts.

Progesterone inhibits the secretions of the cervical epithelia producing scanty, viscous, opaque mucus with low spinnbarkeit and absence of ferning (Zondek and Rozin, 1954; Roland, 1962; Mac Donald, 1969; Cribor et al, 1970; Moghissi et al, 1972; Elstein, 1978). Water content is 92-94%, cellularity increases. The pH of cervical mucus though never acidic changes to a more alkaline level in the ovulatoray phase and it is also altered chemically so as to acquire a considerable content of mucoprotein and polysaccharide. All the acquired features result in a medium that is admirably suited to sperm migration.

The Odeblad Concept

Early investigations by Odeblad (1973) using nuclear magnetic resonance and other physical techniques enabled him to propose a hypothesis which explained the unique rheological properties of cervical mucus, which is composed of a high and low viscosity component. In this concept, he stated that the high viscosity component of the cervical mucus was made up of long flexible macro-molecules (Fig - 1) which were joined up in bundles which he called "micelles", which averaged about 0.5 um diameter. These glycoprotein molecules had their "water of hydration" bound up around them which contributed to their structure. In between these "miscelles" he suggested there were cavities of free "water" up to 10 um diameter, which under

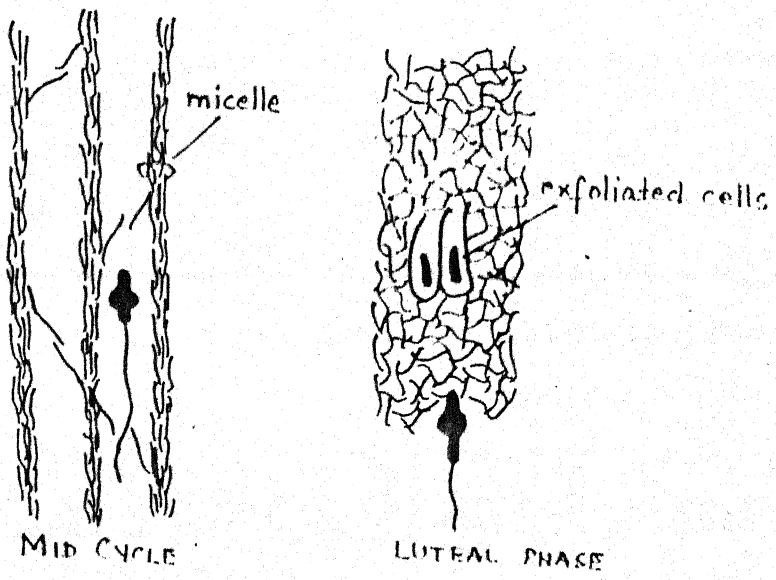


FIG. 1. THE MOLECULAR STRUCTURE OF CERVICAL MUCUS AT MIDCYCLE AND IN LUTEAL PHASE.

oestrogenic stimulus increase in size and under the influence of progestogens became very much smaller. Thus when there was progestational influence the bonding between the glycoprotein macromolecules alters to form a finer meshwork (0.3 u) which prevents entrance of the sperm. However, when the bundles are larger and the "micelles" are forming, there are free elements between the high viscosity components which facilitate the transmission of sperm through the acqueous phase.

Odeblad and his group (1978) have now proposed a "dynamic mosaic model" of the human ovulatory cervical mucus (Fig - 2). Cervical mucus has a heterogenous nature. The more viscid parts, which they call EL mucus lie as "loafs" in a more fluid material. After drying, the "loafs" are easily recognised in the ferning material by their large crystals with a flower like arrangement. Also, the distribution of sperm in the cervical mucus at post-coital or in vitro sperm penetration testing, shows a heterogenous distribution. Progressively moving sperms are usually confined to narrow high ways of more fluid mucus which they call "strings" consisting of ES mucus. They propose a 3- dimensional mosaic pattern, with a high degree of order, inside the cervical canal; it comprises these "loafs" and "strings" which are being continually altered to form a dynamic mosaic. The arrangement of this mosaic may be important for effective upward sperm progression, since

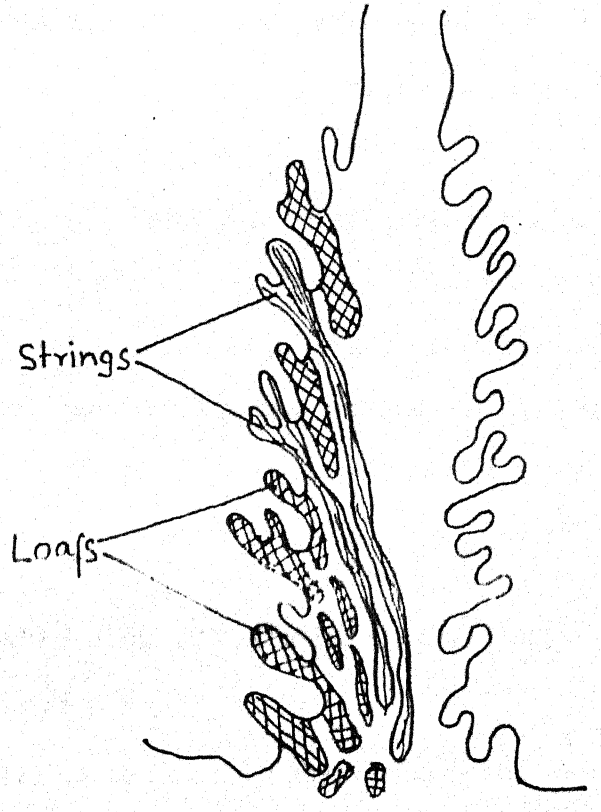


FIG. 2. CERVICAL MUCUS SHOWING STRINGS AND LOAFS.

only complete "strings" of ES material have the capacity to convey the sperm rapidly upwards in the cervical canal. This ES material is identical with the previously described type E mucus (oestrogenic mucus) Fig- 3. At mid-cycle, the crypts, responsible for the production of "strings", are most abundant in upper part of the cervical canal. The whole system is not static but dynamic and undergoes continuous changes with time. After invading a "string" sperm rapidly (within a few to thirty minutes) progress to some crypts, which serve as a sperm reservoir with an average storage time of about fifteen hours. Some sperms may rapidly advance directly into the uterine cavity, probably utilising "staircases" made up of fused ES fragments.

Biochemical basis for the changing physical properties of Human Cervical Mucus

It has been established that the visco-elastic properties of the mucus are derived solely from a high molecular weight glycoprotein in the mucus termed the mucus glycoprotein or mucin. This compound has been visualised by scanning electron microscopy (Daunter et al, 1976) and seen to form a network of filaments or membranes whose pore size is maximal at mid-cycle. It is at this level that variation in the mucus rheology is governed.

Three groups of experimental evidence are there which attempt to link chemical changes in the mucus glycoprotein to its variable visco-elastic properties.

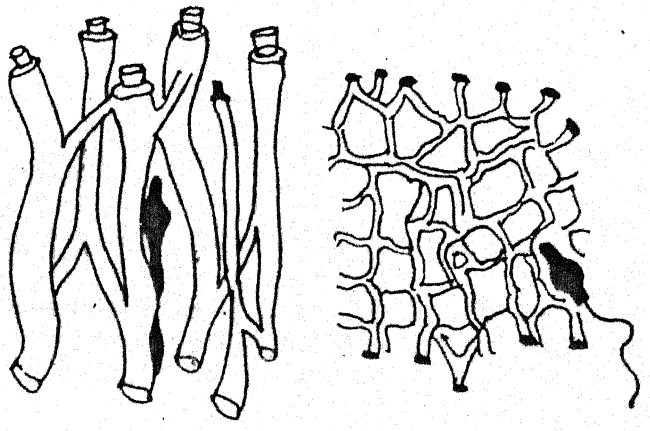


FIG. 3. SCHEMATIC 3-DIMENSIONAL VIEW OF TYPE E AND TYPE G MUCUS.

1. Changes in the degree of cross linking between adjacent glycoprotein molecules, reducing the freedom of movement of the macromolecular complex.

2. An increase in the net charge of the glycoprotein such that similarly charged groups are brought into sufficient proximity to cause electro-static interaction resulting in a decrease in the flexibility of the aggregate.

3. A change in the degree of hydration of the glycoprotein directly affecting its visco-elasticity by a simple concentration effect.

The presence of cross linking proteins which are structurally distinct from the cervical glycoprotein was suggested by Gibbons (1969). Such proteins could act as bridges between adjacent glycoprotein molecules and consequently their concentration would alter during the cycle to produce the cyclic rheology.

The second proposed mechanism for the variation in mucus rheology is based on an alteration in the charge distribution within the aggregate of glycoprotein molecules. The glycoprotein is known to contain charged groups which include sulphated carbohydrates, the carboxyl group of N-acetyle neuraminic acid (SIALIC ACID) and the charged amino acids of the protein core. The charge on these groups

could be modified by variation in the pH or the ionic strength of the mucus but neither of these change sufficiently during the menstrual cycle to alter the degree of ionisation.

Analysis of pooled pre and post-ovulatory human mucus has shown that there is variability at the level of the carbohydrate part of the glycoprotein while the protein core is unchanged, and that this is restricted to the two terminal saccharides, fucose and N-acetyle Neuraminic acid (Iacobelli et al, 1971). It has previously been shown that these two carbohydrates are inversely proportional in epithelial glycoproteins (Dische, 1963) and this type of variation was also found between the pre-ovulatory mucus in which fucose predominates and post-ovulatory mucus which has elevated levels of N-acetyl neuraminic acid (Chantler and Debruyne, 1977).

The third theory, of Wolf et al (1977), relates the cervical mucus rheology to the degree of hydration of the mucus glycoprotein. Using re-constituted mucus, this group has shown that the whole range of visco-elasticity seen during the normal menstrual cycle can be obtained simply by altering the degreeof hydration of the glycoprotein.

Doehr, S.A. and Moghissi, K.S., 1973, described that the principle constituent of cervical mucus is a

glycoprotein of mucin type which contains a high percentage of carbohydrates (75% - 80%) in contrast to only 20% - 25% amino acid residues. It is thought that carbohydrate side chains occur frequently along the polypeptide chains in this type of glycoprotein and are covalently bound to it by an O-glycosidic linkage.

The components of oligosaccharide side chains are N-acetyl galactosamine, N-acetyl glucosamine, galactose, fucose and sialic acid. Sialic acid is present in the form of N-acetyl neuraminic acid in human mucin and N-glycocyl in bovine mucin and it invariably occupies a terminal position (Gibbons, 1967). The functional role played by the sialic acid is of considerable interest. At physiologic pH, sialic acid is negatively charged owing to the presence of carboxyl group. Because the sialic acid residues are always terminally positioned on the oligosaccharide side chain, they are in a position to repel one another, hereby contributing to a distension of the coil.

This leads to a reduction of flexibility and an increase in viscosity. Viscosity is the most important property of cervical mucus relative to sperm penetration. A highly viscous mucus forms an effective barrier to sperm penetration.

Changes in the ratio of sialic acid to fucose might be a major contributing factor to cyclic changes in physical properties of cervical mucus.

Same workers (Seguy and Simmonet, 1933) have shown that there are cyclic physico-chemical changes in the cervical mucus of normally menstruating women. These changes are under the influence of oestrogen, which seems to render the cervical mucus more receptive to the spermatozoa at the time of ovulation. After ovulation, progesterone exerts an inhibitory effect on the production of cervical mucus and an increase in the content of sialic acid.

Zondek in 1954 showed that the cervical mucus was a more sensitive indicator of oestrogenic activity than the vaginal epithelium. At the time of ovulation certain significant changes occur in the character of the cervical mucus facilitating the penetration of the sperms readily (Lamer et al, 1940; Bergman, 1950).

The mucus from the cervical canal seems to be of significance in many aspects of reproductive physiology; the primary function being protection of uterine cavity and to control sperm penetration through the cervix. The production of mucus, as well as certain changes in its physical properties depends upon the hormonal situation.

Inspite of the well documented modifications in pre and post ovulatory mucus controversies existed regarding the underlying biochemical changes. Schwartz, 1954, 1955; Botella- Llusia, 1960, suggested that the structural elements of the mucus gel consisted of long, thread-like glycoprotein molecules with a high degree of branching and physical properties of mucus are dependent upon these active molecules and biochemical alteration in its content during the menstrual cycle may have effects upon the spermatozoal penetrability through changes in viscosity.

The only report in this subject was given by Gibbons, R.A., 1959 on bovine mucus, who found a somewhat lower sialic acid content in oestrus mucoid with respect to that of pregnancy. (Dische, Z, and Shettles determined the sailic acid according to the method of Aminoff, after hydrolysis of the sample in 0.1 N sulphuric acid for 30 minutes. N-acetyl neuraminic acid (Sigma Chemicals Co., St. Louis, M.O.) was used as standard.

Although marked physical changes in cervical mucus occur during pre-ovulatory, post-ovulatory and pregnancy period, the study of samples from these periods showed similar composition with respect to protein, hexose, fucose and sulphate. Elstein found that sialic acid exhibited a significant variation with respect to the period of the sampling (30 ug/mg in pre-ovulatory to 49 ug/mg in pregnancy.

Several observations were made indicating that decrease of this substance in cervical mucus in the ovulation period is associated with a parallel increase in sperm penetrability.

It has been suggested (Gibbons, 1959) that the differing physical properties of epithelial mucin are related to the expansion factor of the constituent thread-like random coil molecules. The pregnancy and oestrous mucoid have markedly different expansion factor (Gibbons & Glover, 1959) and it is probable that this in turn is related to the sialic acid component, since the sialic acid content constitutes the only major integral component and chemical distinction between the two mucoids so far known. They found that the sialic acid content of the pregnancy mucoid (17.5%) is higher than that of oestrous mucoid (14%) and the results of hydrolysis of oestrous mucoid suggest that part of the sialic acid is present as end group and that the sialic acid content is one of the factors affecting the physical properties of mucin.

Cervical mucus is the main factor involved in regulating sperm migration. These observation are in agreement with the findings of Odeblad (1962) who showed that there are certain undulations in the mucoprotein molecules which produce moving spaces for the migration of spermatozoa under the effect of sialic acid and fucose.

Odeblad (1959, 1969) found in this experiments that the highest penetration rate was as a rule present when the production of cervical mucus was intense and homogenous as found during the ovulatory phase. There is also considerable evidence that the mucoid molecules of mucus during the influence of oestrogen are present in parallel micelles between which the sperms migrate.

This evidence was supported by Gibbons and Glover, 1960; Tampion & Gibbons, 1962; Sobrero, 1963; Odeblad, 1968; and Gibbons and Mattner, 1966. They found that the cervical mucus obtained during the ovulatory period if drawn into a capillary tube, the mucoid molecules arrange themselves in parallel with the axis and the spermatozoa migrate parallel to the glass wall and towards the other end of the capillary tube.

During the normal menstrual cycle the glandular cells of the cervix are subjected to fluctuations in hormonal levels in the blood, mainly oestrogens and progesterone. This is reflected in variations in quality and quantity of cervical mucus. Sialic acids have been shown to be part of the mucoproteins and are located in an end position (Gottschalk, 1963; Dische, 1963; Sobrero, 1963). They are the first moieties to be cleaved off the molecule during hydrolysis. There is substantial evidence that the cervical mucus consists of long thread like molecules of

glycoprotein with cross linkages occuring via the sialic residues of the glycoprotein. The hormonal dependence of the sialic acid concentration in the mucus was shown by studying the mucification in the mouse vagina (Carlborg, 1966). Oestrogens were shown to decrease the content of sialic acid in the mucoproteins, whereas progesterone alone left it unchanged. However, an optimal combination of oestrogen and progesterone caused a marked incorporation of sialic acids in themucoproteins. Characteristic variations were also recorded during the oestrous cycle, pregnancy and pseudopregnancy.

Carlborg, Mc Cormick & Gemmell, 1968 studied the sialic acid concentration in cervical mucus during nine ovulatory cycles in 5 normally menstruating women (age 23-32 years) at the Worcester foundation for experimental Biology, U.S.A. and found that in all 9 ovulatory cycles, a mid cycle fall in sialic acid concentration occured, the mean being on day 16 designated as day 0 and the days preceeding were numbered -1, -2 etc and the days following as +1, +2 etc, Fig 4 of their publication shows that a continuous decrease in sialic acid concentration occured reaching the lowest value of 20-25 ug/mg of dry weight of cervical mucus on day 16, followed by a rapid increase. Thus sialic acid concentration decreases during the proliferative phase of the menstrual cycle and increases during

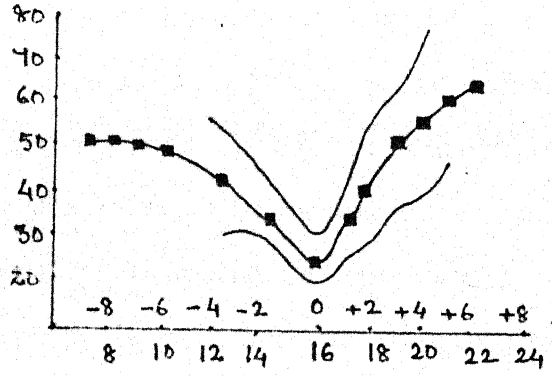


FIG. 4. CONCENTRATION OF SIALIC ACID IN CERVICAL MUCUS (Curve of 9 ovulatory cycles studied by Carlborg, Mc Cormick and Gemzell, 1968.).

the secretory phase. The temperature curves obtained were all biphasic and the thermal shift on the average was simultaneous with the lowest sialic acid concentration.

They analysed the sialic acid concentration and maximum sperm receptivity and presented them together. In 14 cycles, a post ovulatory increase in sialic acid concentration was found while in 4 non-ovulatory cycles, no change in the sialic acid concentration was noticed. Three cycles could not be studied because of insufficient material of cervical mucus. In two cycles no post-ovulatory increase was noticed. Cyclic changes in sialic acid concentration were found together with a positive sperm receptivity in 12 cycles. In 7 cycles, absence of cyclic changes in sialic acid concentration was found together with a lack of sperm receptivity.

Carlborg, Johansson & Gemzell, again in 1969, studied the sialic acid content and sperm penetration of cervical mucus in relation to total urinary oestrogen excretion and plasma progesterone levels in 6 ovulatory women with regular menstrual cycles. In four women of known fertility and in one normally menstruating, women, the peak in sperm penetration and the 'dip' in sialic acid content occured almost simultaneously with the ovulatory oestrogen peak in urine and preceeded the probable

day of ovulation by one day. In one women who was barren for several years, the steroid patterns were normal but the peak in sperm penetration and the dip in sialic acid content occured one day after ovulation. It is generally thought that the changes in the cervical mucus during the folicular phase are due to oestrogens, while in the luteal phase, they are dependent on the superimposed effect of progesterone. With regard to oestrogens, Carlborg and Gemzell (1969) who treated an ovulatory women with gonadotrophin (HPG) found that the sialic acid content and sperm penetration of the cervical mucus could be related to changes in the excretion of urinary oestrogens.

Cervical mucus is a heterogenous secretion and the most important constituent is a hydrogel, rich in carbohydrate, consisting of at least two electrophoretically defined glycoproteins of the mucin type (Moghissi & Syner 1970, Schumacher and Moghissi, 1973) and the varried constituents undergo apparent changes in its concentration around the time of ovulation. Variations in the structure and viscoscity of these macromolecules are responsible for the cervical secretions periodic receptivity to and inhibition of sperm penetration. Under the effect of progesterone the secretion becomes scanty, highly viscous and cellular. The ferning disappears and spinnbarkeit is greatly

reduced due to extensive crosslinking between mucin strands. The normal pre-ovulatory decrease in albumin and sialic acid and the increase in mucins are altered. The pH however is not significantly changed.

Saliva is the most accessible of the body fluids and periovulatory changes in its composition have been recognised. Oster and Yang (1972) reported that while alkaline phosphatase, aryl sulphatase, N-acetyl beta-D glucosaminidase, peroxidase and phosphate are increased the sialic acid content of whole saliva is at a minimum at mid-cycle. Saliva from normally menstruating women has been reported to exhibit a ferning pattern (arborization) in the proliferative phase of the cycle which disappeared in the secretory phase. This cyclic ferning phenomenon, although clear in the case of cervical mucus is not pronounced in the case of saliva. The ferning phenomenon of saliva and of cervical mucus is associated with the physiochemical properties of mucopolysaccharides, which in turn are governed by the sialic acid content of the negatively charged macromolecules. The cyclic change in sialic acid is reinforced by the fact that it occurs in subjects close to the occurence of mittelschmerz. If mittelschmerz coincides with ovulation, the decline in sialic acid anticipates ovulation by 1-2 days.

It was reported by Moghissi et al, in 1976, that the sialic acid concentration of mucus decreases during the proliferative phase, reaches a minimum at the time of ovulation and rises subsequently. Cyclic changes in the various physical properties and biochemical constituents of the mucus are known to reflect accurately the hormonal changes associated with an ovulatory menstrual cycle and may be utilised clinically to determine the time of ovulation. There is a significant increase in the amount, crystallisation and spinnbarkeit property and a marked decrease in viscosity and cell content of the mucus in the mid-cycle prior to ovulation and a reversal of these changes following ovulation and in the luteal phase. Among various constituents, albumin, alpha-1-antitrypsin, transferrin, Ig G, Ig A and sialic acid decrease, whereas the relative percentage of mucin increases in the mid-cycle.

MATERIAL & METHOD

MATERIAL AND METHODS

The present study was conducted in the Department of Obstetrics and Gynaecology, M.L.B. Medical College and Hospital, Jhansi in collaboration with the department of Biochemistry, M.L.B. Medical College, Jhansi, over a period of one year for the assessment of "Sialic acid" content of the cervical mucus in relation to female infertility.

SELECTION OF PATIENTS

Women of reproductive age group, who were attending the outdoor patient department of the above mentioned hospital were included in this study. They were divided into the following groups :-

CONTROL GROUP :

Healthy volunteer fertile women as control who were devoid of any organic disease, irrespective of parity, with known L.M.P.

EXPERIMENTAL GROUP :

Women with clinically diagnosed primary infertility (i.e. having not conceived upto 2 years of married life), having no attributable cause to infertility and with husbands

having normal fertility status. Patients were of known L.M.P. and regular menstrual cycles and not taking any contraceptive measure.

A detailed history was taken, clinical evaluation was done consisting of general, physical, systemic, P/S and P/V examinations.

METHOD OF SAMPLE COLLECTION :

Samples of cervical mucus were collected during selected periods of menstrual cycle i.e. 15 ± 2 days of the cycle (ovulatory mid cycle) in both the control and the experimental group.

Patient was laid in lithotomy position. Part was painted and draped. A sterile unlubricated speculum was used and the anterior lip of cervix caught hold of by a volsellum. They area around the external os was wiped with absorbent dry cotton-wool. Mucus was aspirated with an insulin syringe (without needle). The nozzle of the syringe was inserted as high as possible into the endocervical canal and withdrawn until the external os was reached and the suction then released to avoid contamination of the endocervical specimen with vaginal contents. It was difficult to separate the upper from the lower contents of the endocervical canal so the entire specimen was examined. Repeated aspirations were made. Specimens tinged with blood were discarded.

The mucus was collected in a sterile test tube and stored with 0.2 ml of distilled water, in a deep freeze at -18°C within an hour of collection until the sialic acid analysis could be made. Mucus was carefully mixed with a glass rod before analysis. Cervical mucus was studied under the following headings :-

(A) Study of the physical properties

(B) Cervical mucus grading

(C) Post-coital test

(D) Sialic acid analysis

(A) Study of the Physical Properties :

Following physical properties of the cervical mucus were studied at the time of collection of the sample, along with the biochemical estimation of the sialic acid.

(1) Appearance : Appearance of the cervical mucus was noted as being -

Transparent	-	Crystal clear
Clear	-	Resembling the white of raw egg.
Turbid	-	Whitish
Cloudy	-	Still whiter

(2) Quantity : Quantity was graded as 1+ to 4+

1 + = 0.1 ml or less

2 + = 0.2 ml

3 + = 0.3 ml

4 + = 0.4 ml or more

(3) Viscosity : Because of the small quantities involved, the viscosity was evaluated relatively in terms of 1+ to 4+.

1 + - Normal mid cycle mucus

4 + - Thick, viscous premenstrual mucus

(4) Spinnbarkeit (Spinability) : The length of the mucus thread stretched between a glass slide and coverslip was measured in centimeters immediately after collection of the sample.

(5) pH : The pH was determined with universal indicator pHydrion paper of wide range pH 2-10 of Sarabhai M Chemicals, India by placing a drop of cervical mucus from the collecting syringe.

(6) Fern test (Arborization, P.L. reaction) : Ferning was recorded as -

1 + - linear (minimal degree of oestrogen effect)

2 + - having some palm leaf appearance with arborization of the leaves at 90° to each other.

3 + - having moderate degree of arborization when the palm leaf appearance involves angulation at three right angles.

4 + - showing maximal arborization where the palm leaves appear at four right angles to each other.

(B) CERVICAL MUCUS GRADING :

Cervical mucus grading was done according to the system outlined by Brown in 1973. The day on which the patients had the maximum amount of clear mucus was taken as the day of maximum mucus grading (MMG) and as her interpretation of the day of ovulation. Where equal score occured on two days, the second of these was taken as the maximum.

The ovulation or fertile phase of the cycle was defined as the day when the mucus grade was 5 or more.

CERVICAL MUCUS GRADING

Type	Grading	Description
	-1	Dry sensation
	Definite change	
	1	Not dry, nothing seen
Infertile	2	Yellow or white, minimal
	3	Yellow or white, sticky
Possibly fertile	4	Cloudy, becoming clearer sticky.
	Definite change	
	5	Thimer, more stretchy
Fertile	7	Stretchy, lubricative, clear,
	9	wet, slippery, variable amount.

(C) POST-COITAL TEST (SIMS-HUHNER TEST) :

This test was conducted at the time of ovulation. Samples were collected post-coitally with prior abstinence of at least three days, within 2-8 hours, from the endocervical canal and posterior vaginal fornix separately. Spermatozoa were studied for the following :-

1. Number : of spermatozoa in the vagina and whether they have invaded the cervical mucus.

2. Extent of motility : This was recorded as
   - progressively or actively motile
   - having localised motility
   - feebly motile or sluggishly motile
   - immotile

3. Morphology of spermatozoa : Normal or abnormal (double head, double tail, no head, no tail, extra long tail, extra short tail, extra large head, very small head, abnormal shape).

4. Clumping - if at all present.

The cases included under the study were those who had at least 40 million sperms per ml of semen, at least 40% of which showed good progressive motility, all sperms were morphologically normal and no clumping was seen. Mac Leod considers that the normal sperm count is 40 millions/ml

or 125 million total per ejaculate and true oligospermia is represented below 20 millions/ml. The post-coital test was interpreted as follows :-

Negative PCT - No penetration of mid-cycle cervical mucus by sperms.

Unsatisfactory or poor - Less than 6 sperms per high power field with no or poor motility between 2 to 6 hours after coitus.

Satisfactory - More than 6 sperms per high power field, progressing purposefully in clear, ductile plentiful mucus within 8 hours of coitus.

(D) QUANTITATIVE ESTIMATION OF SIALIC ACID (N-ACETYL NEURAMINIC ACID) NANA :

Sialic acid was estimated by the thiobarbituric acid method (Warren, 1959 a,b) which was further modified by Aminoff (1959).

Principle : N-acetyl neuraminic acid is oxidised with periodate, resulting in the formation of chromogen. Oxidation with periodate is more rapid at 37°C than at room temperature, and the final colour intensity is dependent on both the pH and the period of oxidation. The excess of periodate is best removed with acid arsenite.

The intensity of colour obtained on heating with thiobarbituric acid is independent of the pH of the reagent over the range of 7-10.

The coloured complex is both more stable and more soluble in acidified but-an -1-01. The absorption peak of the coloured material is sharper at 549 mu and more intense in the butan -1 - 01 than in the acqueous phase before extraction.

Reagents :

(a) Periodate reagent : 25 m M periodic acid in 0.125N H_2SO_4 (pH = 1.2).

(b) Sodium arsenite reagent : 2% solution of sodium arsenite in 0.5 NHcl.

(c) Thiobarbituric acid reagent : 0.1 M solution of 2-thiobarbituric acid in water, adjusted to pH 9.0 with NaOH. It keeps well for about a month in dark bottle of $4^{\circ}C$.

(d) Acid butanol : Butan - 1 - 01 containing 5% (V/V) of 12 NHcl.

(e) Standard of N-acetyl neuraminic acid :
1 mg of N- acetyl neuraminic acid (from E.coli, Sigma 98f crystalline, Anhydrous Mol. Wt. 3093 (M/S Sigma Chemicals, U.S.A.) was dissolved in 10 ml of distilled water. The final concentration of standard was 100 ug/ml.

(f) Sample of cervical mucus - Cervical mucus was diluted with distiled water upto 1:10.

Procedure :-

A solution of the sample, blank or standard (containing 5-50 ug of N-acetyl neuraminic acid) in 0.5 ml of water is treated with 0.25 ml of the periodate reagent for 30 min in a water bath at 37°C. The excess of periodate is then reduced with 0.2 ml of the sodium arsenite. As soon as the yellow colour of the liberated iodine has disappeared (1-2 min), 2.0 ml of the thiobarbituric acid reagent is added and the test sample is covered and heated in a boiling water bath for 7.5 min. The coloured solution is then cooled in ice water and shaken with 5.0 ml of the acid butanol. The separation of the two phases is facilitated by a short, rapid centrifuging and the intensities of the colours in the butanol layer are compared at 550mm using green filter in a colorimeter.

PROTOCOL

Reagent	Test	Standard	Blank
Cervical mucus (diluted (1:10)	0.5 ml	-	-
Standard (100 ug/ml)	-	0.5 ml	-
Distilled water	-	-	0.5 ml
Periodate reagent	0.25 ml	0.25 ml	0.25 ml
	(Treated for 30 min in a water bath at 37°C)		
Sodium arsenite	0.2 ml	0.2 ml	0.2 ml
	(As soon as yellow colour of iodine disappeared) (about 1-2 min)		
Thiobarbituric acid	2.0 ml	2.0 ml	2.0 ml
	(Heated on boiling water bath for 7.5 min and then cooled in ice cold water).		
Acid butanol	5.0 ml	5.0 ml	5.0 ml
	(Shaken well and centrifuge to separate and layers).		

Intensities of colours in the butanol layer are compared at 550 mu in a colorimeter. Take reading within 2 hours.

Calculation :

N-acetyl neuraminic acid in cervical mucus

$$= \frac{\text{O.D. of Test}}{\text{O.D. of standard}} \times 100 \times \text{Concentration of standard} \times \text{Dilution factor}$$

$$= \frac{T}{S} \times 100 \times 100 \times 10 \text{ ug/100ml} = \frac{T}{S} \times 1000 \text{ ug/ml}$$

O.D. = Optical Density

Choice of Method :

The method of Aminoff was chosen as a material of estimation of NANA in sialo mucoids because the thio-barbituric method has an advantage of detecting both the free and the bound form of sialic acid residues in the cervical mucus.

OBSERVATION

OBSERVATIONS

The study was done on 10 control patients and 50 experimental patients chosen from the ones attending the outdoor patient department of the Department of Obstetrics and Gynaecology. Properties of the cervical mucus were studied and comparison was done between the control and experimental groups. Observations were evaluated as under.

TABLE NO. I

COMPARISON OF QUANTITY OF CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

Cases	No. of samples	QUANTITY							
		0.1 ml		0.2 ml		0.3 ml		0.4 ml	
		No.	%	No.	%	No.	%	No.	%
Control	10	0	0	6	60	3	30	1	10
Experimental	50	19	38	26	52	5	10	0	0

Table No. I shows the quantity of cervical mucus ranging from scanty (0.1 ml or 1+) to abundant (0.4 ml or 4+). In the control group the quantity obtained was 0.2 ml or more. Only one patient had an abundant quantity. In the experimental group, majority had 0.1 ml or 0.2 ml i.e. scanty to moderate. None had an abundant quantity.

TABLE NO. II

COMPARISON OF VISCOSITY OF CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

Cases	No. of samples	VISCOSITY					
		Low		Moderate		High	
		No.	%	No.	%	No.	%
Control	10	7	70	3	30	0	0
Experimental	50	24	48	21	42	5	10

Table No. II shows that 70% control cases had a low viscosity and 30% had moderate viscosity. None had a high viscosity. In the experimental group 5 i.e. 10% cases had a high viscosity in the ovulatory period where as the remaining had a low or moderate viscosity.

TABLE NO. III

COMPARISON OF SPINABILITY OF THE CERVICAL MUCUS IN THE CONTROL AND EXPERIMENTAL GROUPS.

Cases	No. of samples	SPINABILITY							
		$\angle$ 6 cms		7-10 cms		11-14 cms		$\nearrow$ 14 cms	
		No.	%	No.	%	No.	%	No.	%
Control	10	0	0	2	20	7	70	1	10
Experimental	50	3	6	30	60	16	32	1	2

Table No. III evaluates that the cervical mucus in control group was stretchable betwee 7-14 cms, though one case had a spinability $\nearrow$ 14 cms, but in experimental cases about 6% had a poor spinability and the remaining had an adequate spinability.

TABLE NO. IV

COMPARISON OF pH OF CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

Cases	No. of samples	pH					
		Acidic ($\swarrow$ 7.0)		Neutral (7.0)		Alkaline($\nearrow$ 7.0)	
		No.	%	No.	%	No.	%
Control	10	1	10	4	40	5	50
Experimental	50	12	24	24	48	14	28

The above table shows that 50% cases of the control group had an alkaline pH, 40% had neutral and only 10% cases had an acidic pH, where as in experimental group, 48% had a neutral pH, 28% are alkaline and 24% acific pH of the cervical mucus.

TABLE NO. V

COMPARISON OF THE FERNING PROPERTY OF THE CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

Cases	No. of samples	FERNING (ARBORIZATION)							
		+4		+3		+2		+1	
		No.	%	No.	%	No.	%	No.	%
Control	10	6	60	4	40	0	0	0	0
Experimental	50	11	22	29	58	8	16	2	4

Table No. V shows that ferning was of high grade i.e. +4 or +3 in the control group. In the experimental group 20% cases showed a poor ferning pattern though the remaining 80% had a good ferning pattern.

TABLE NO. VI

COMPARATIVE CERVICAL MUCUS GRADING IN CONTROL AND EXPERIMENTAL GROUPS

Cases	No. of samples	CERVICAL MUCUS GRADING					
		1-3		4		5-9	
		No.	%	No.	%	No.	%
Control	10	0	0	0	0	10	100
Experimental	50	3	6	6	12	41	82

The above table shows the comparative cervical mucus grading, done at the time of collection of the sample. In control group, all patients had a good grade i.e. more than 5, indicating the fertile phase of the cycle. In the experimental cases, 82% showed a similar grading but 12% had a grade of 4 indicating a possibly fertile phase and 6% showed an infertile phase, though the sample was collected at mid-cycle.

TABLE NO. VII

COMPARISON OF MEAN SIALIC ACID CONCENTRATION (ug/ml of wet mucus) OF MID-CYCLE OVULATORY CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS.

Cases	No. of samples	Mean sialic acid concentration (ug/ml of wet mucus) in mid-cycle ovulatory mucus
Control	10	25.67
Experimental	50	47.32

The above table shows the mean sialic acid concentration of mid-cycle ovulatory mucus in control and experimental groups, the mean being 25.67 ug/ml of wet mucus in control and 47.32 ug/ml of wet mucus in experimental group.

TABLE NO. VIII

RELATIONSHIP OF MEAN SIALIC ACID CONCENTRATION TO QUANTITY OF CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

QUANTITY	CONTROL			EXPERIMENTAL		
	No. of samples	%	Mean S.A.	No. of samples	%	Mean S.A.
0.1 ml	0	0	0	19	38	48.68
0.2 ml	6	60	26.98	26	52	46.10
0.3 ml	3	30	23.73	5	10	48.48
0.4 ml	1	10	23.60	0	0	0

Table No. VIII shows the mean sialic acid and quantity of cervical mucus in control and experimental groups. In the control group, there was an increase in quantity of cervical mucus with a fall in the sialic acid concentration, whereas this pattern was not seen in the experimental group (Graph No. I).

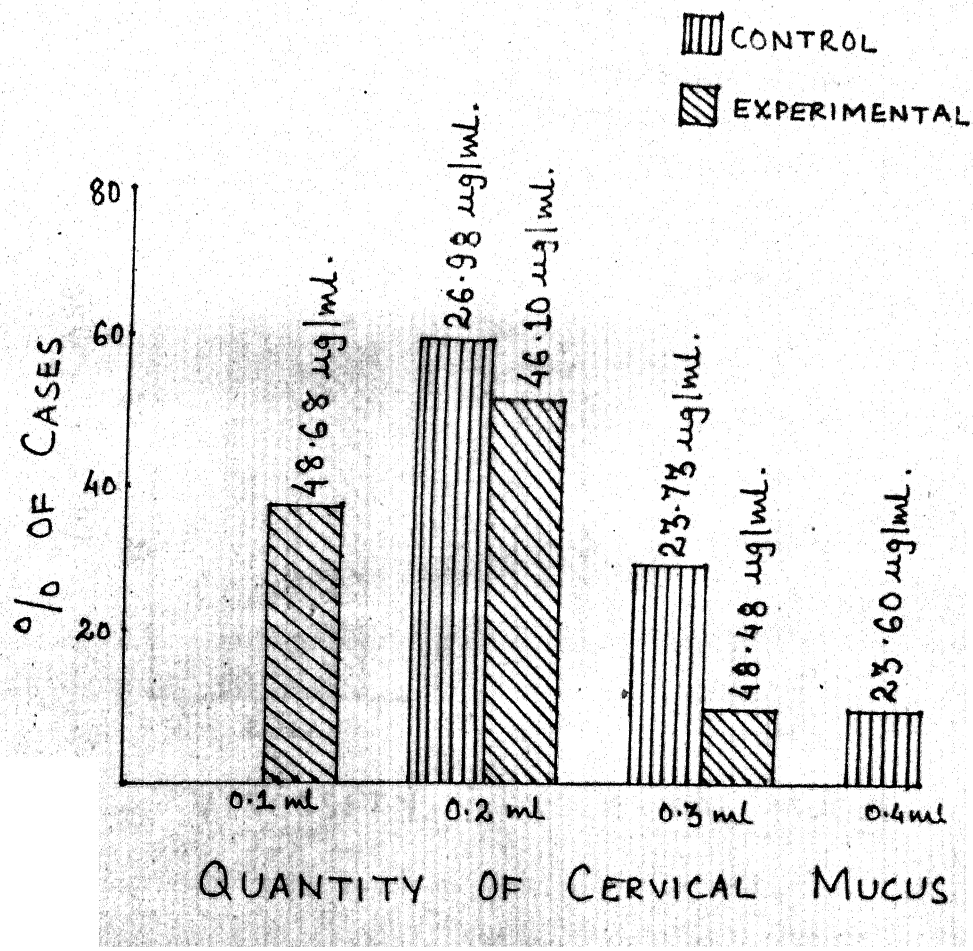
TABLE NO. IX

RELATIONSHIP OF MEAN SIALIC ACID CONCENTRATION TO VISCOSITY OF CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

VISCOSITY	CONTROL			EXPERIMENTAL		
	No. of samples	%	Mean S.A.	No. of samples	%	Mean S.A.
Low	7	70	23.81	24	48	46.82
Moderate	3	30	30.00	21	42	47.03
High	0	0	0	5	10	50.86

The above table shows a direct relationship between the mean sialic acid concentration and viscosity of cervical mucus i.e. lesser the sialic acid concentration, lower the viscosity of the cervical mucus (Graph II).

GRAPH - I. Comparison of Quantity of Cervical mucus in Control and experimental groups vs. Sialic Acid concentration.



GRAPH - II. Comparison of Viscosity of Cervical Mucus in control and experimental groups vs. Sialic acid concentration

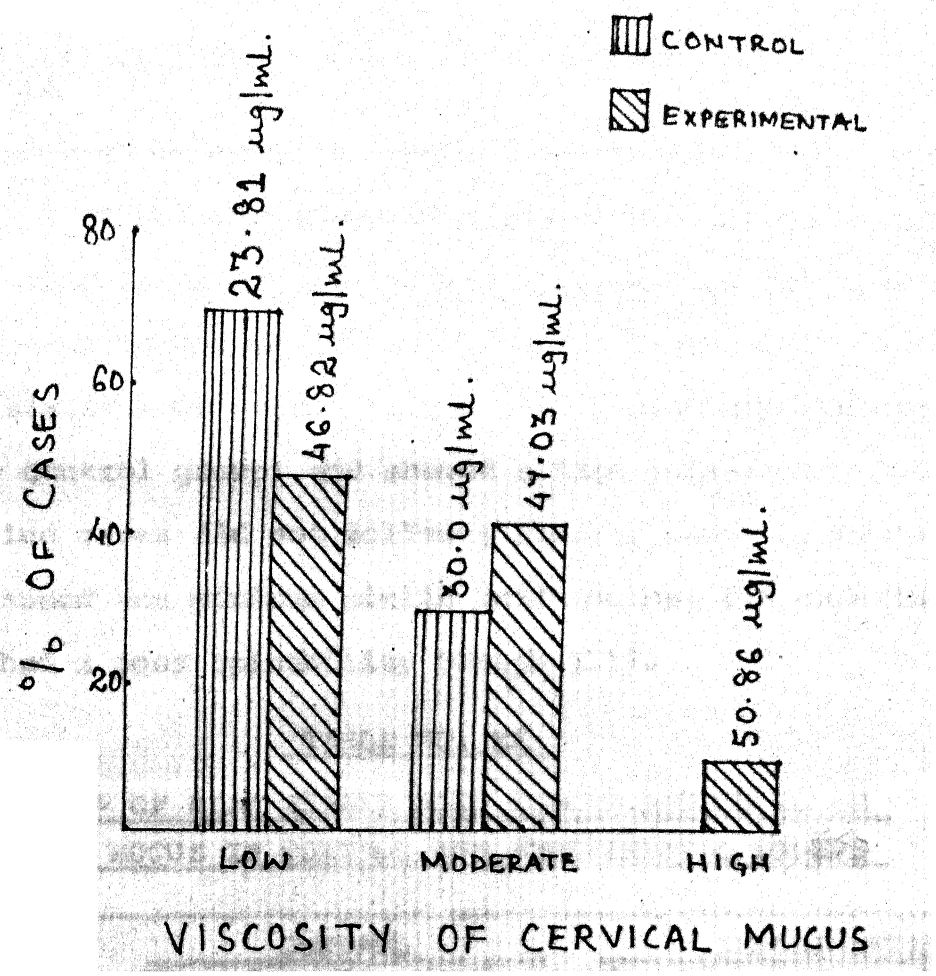


TABLE NO. X

RELATIONSHIP OF MEAN SIALIC ACID CONCENTRATION TO THE SPIN-ABILITY OF CERVICAL MUCUS IN CONTROL & EXPERIMENTAL GROUPS

Spinnbarkeit (Spinability)	CONTROL			EXPERIMENTAL		
	No. of samples	%	Mean S.A.	No. of samples	%	Mean S.A.
∠ 6 cms	0	0	0	3	6	45.43
7 -10 cms	2	20	31.00	30	60	48.23
11-14 cms	7	70	24.95	16	32	47.48
7 14 cms	1	10	20.00	1	2	23.00

This table shows that lesser the concentration of sialic acid in mucus greater the spinability, but this relationship was disturbed in the experimental cases. Only 2% cases had a low sialic acid (equal to the mean sialic acid concentration of the control group) and showed a high spinability but the remaining cases did not follow a similar pattern. 6% cases that showed the minimum sialic acid amongst the experimental cases had a poor spinability (Graph III).

TABLE NO. XI

RELATIONSHIP OF MEAN SIALIC ACID CONCENTRATION TO pH OF CERVICAL MUCUS IN CONTROL AND EXPERIMENTAL GROUPS

pH	CONTROL			EXPERIMENTAL		
	No. of samples	%	Mean S.A.	No. of samples	%	Mean S.A.
Acidic(∠7.0)	1	10	32.00	12	24	51.03
Neutral(7.0)	4	40	25.75	24	48	46.99
Alkaline (7.0)	5	50	24.34	14	28	63.47

GRAPH - III. Comparison of Spinability of Cervical Mucus in control and experimental groups vs. Sialic acid concentration

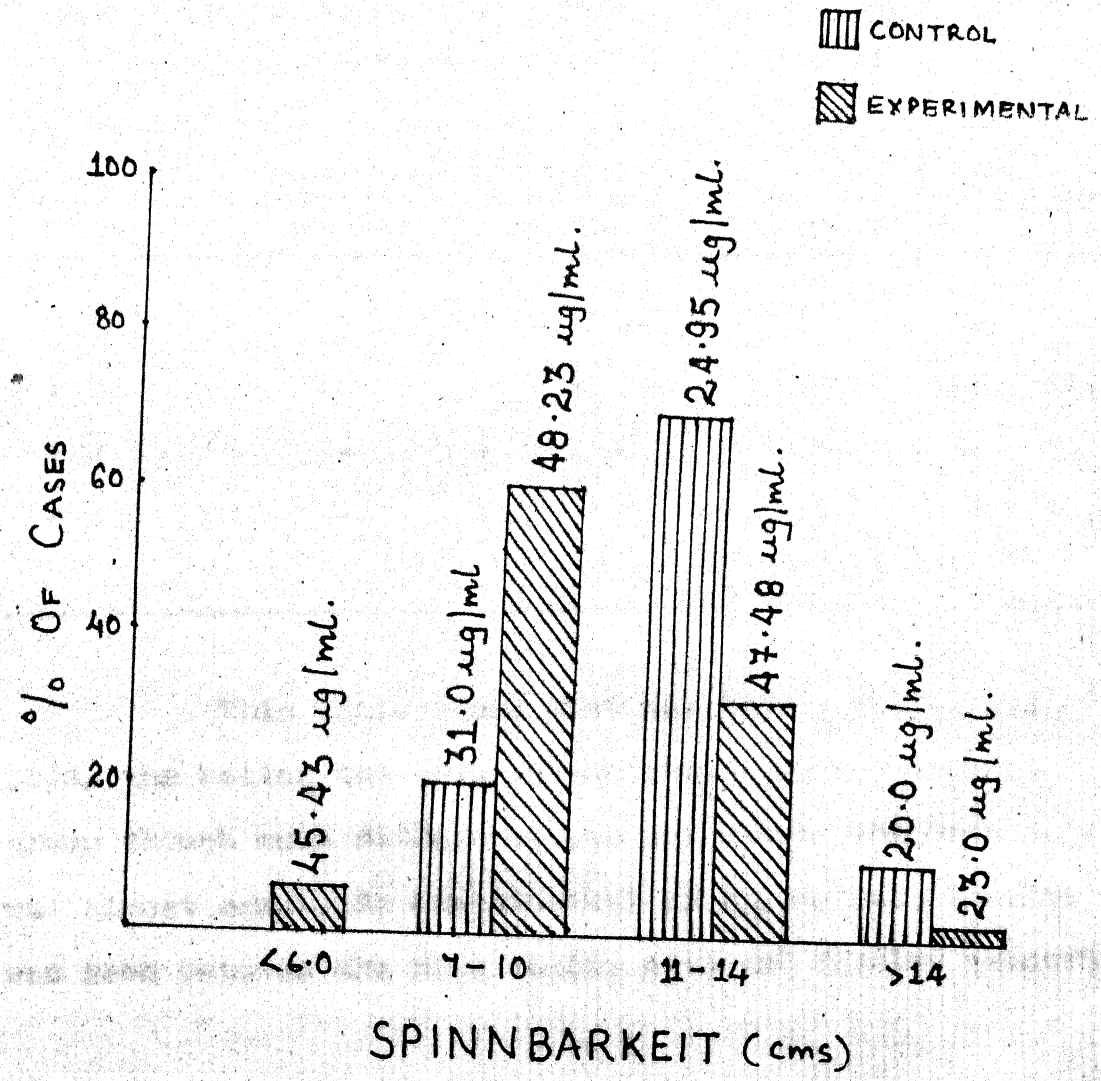


Table No. XI shows that lesser the quantity of sialic acid, greater the pH of cervical mucus i.e. the alkalinity of mucus increases with a falling sialic acid concentration where as no definite relationship could be seen in the experimental group (Graph IV).

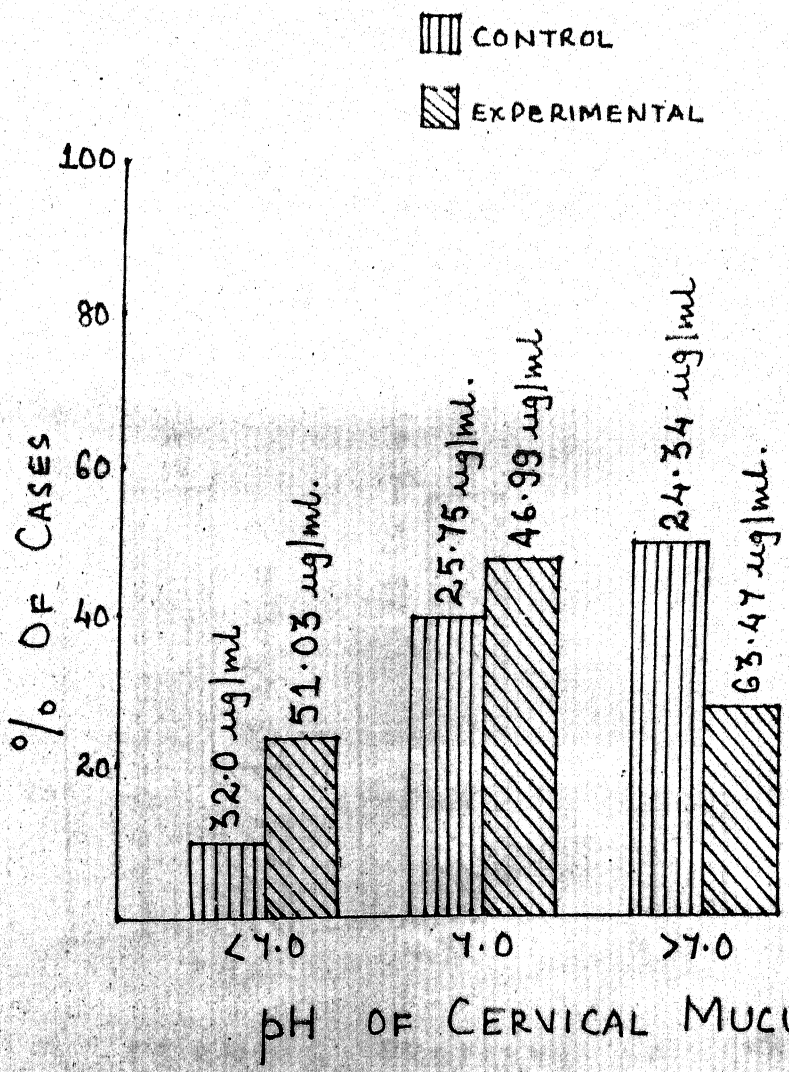
TABLE NO. XII

RELATIONSHIP OF MEAN SIALIC ACID CONCENTRATION TO FERNING PROPERTY OF CERVICAL MUCUS IN CONTROL & EXPERIMENTAL GROUPS

Ferning	CONTROL			EXPERIMENTAL		
	No. of samples	%	Mean S.A.	No. of samples	%	Mean S.A.
+ 4	6	60	28.36	11	22	48.25
+ 3	4	40	28.66	29	58	47.01
+ 2	-	-	-	8	16	36.77
+ 1	-	-	-	2	4	49.15

This table shows that the lesser the sialic acid, the better the ferning phenomenon in the control group though much difference was not noted. The mean S.A. was almost equal. In experimental group, no relationship was seen between the mean sialic acid and ferning property.

GRAPH - IV. Comparison of pH. of Cervical Mucus in Control and experimental groups vs. Sialic acid concentration.



GRAPH - V. Comparison of ferning property of Cervical mucus in Control and experimental groups vs. Sialic acid concentration.

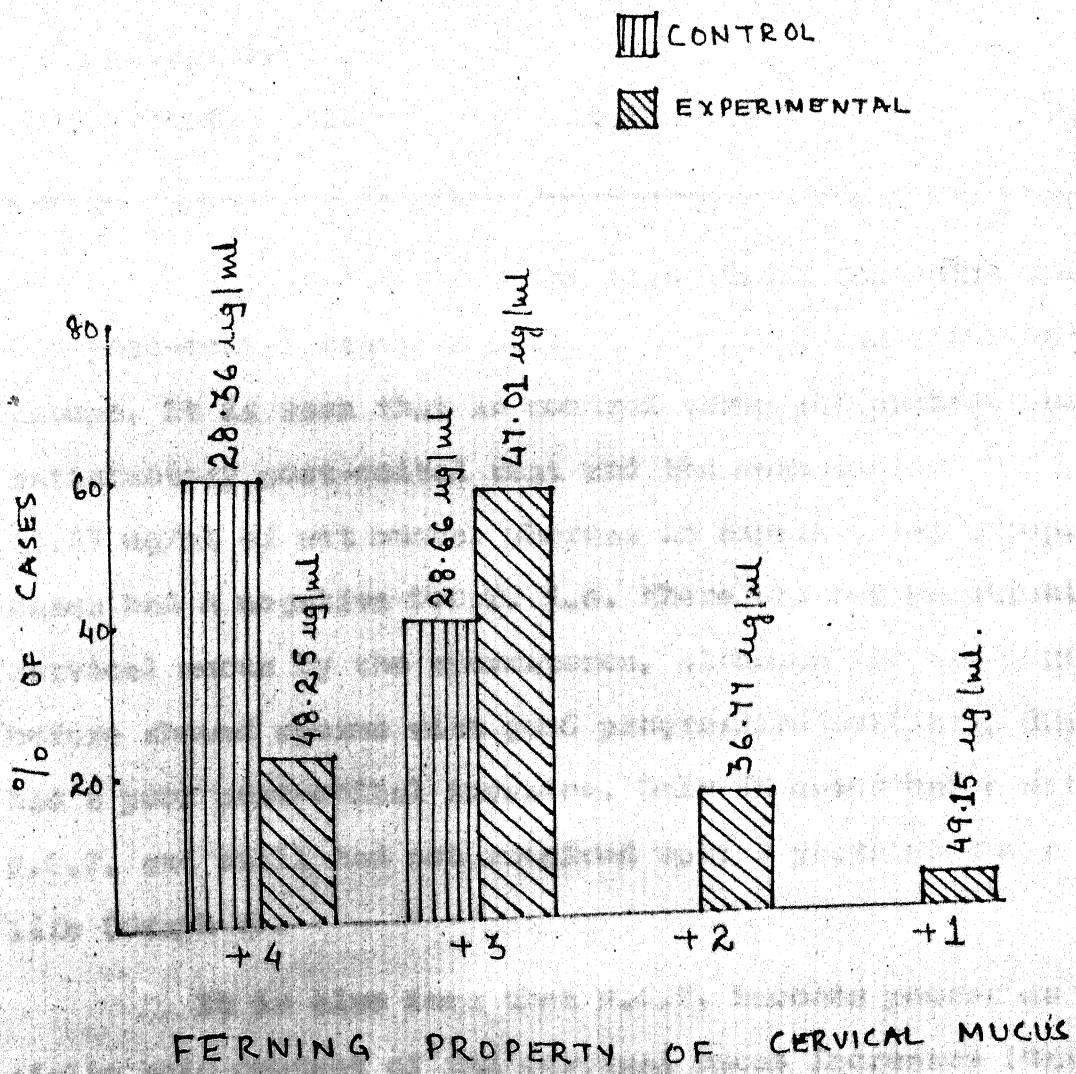


TABLE NO. XIII

RELATIONSHIP OF MEAN SIALIC ACID CONCENTRATION OF CERVICAL MUCUS TO POST-COITAL TEST IN CONTROL & EXPERIMENTAL GROUPS

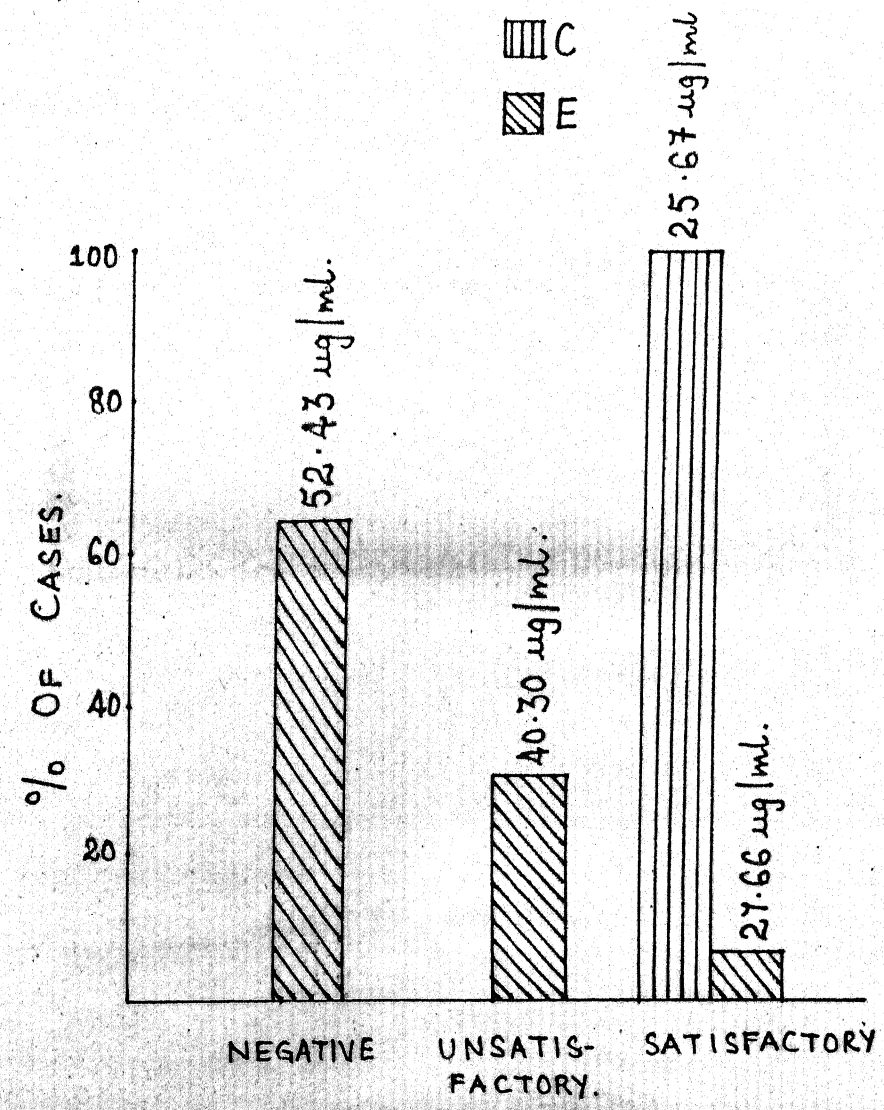
P.C.T.	CONTROL			EXPERIMENTAL		
	No. of samples	%	Mean S.A.	No. of samples	%	Mean S.A.
Negative	0	0	0	32	64	52.43
Unsatisfactory	0	0	0	15	30	40.30
Satisfactory	10	100	25.67	3	6	27.66

This table shows the mean sialic concentration and post-coital tests in both, the control and experimental groups. It is seen that in control group all patients had a satisfactory post-coital test and the mean sialic acid is 25.67 ug/ml of wet mucus, whereas in experimental group, 64% cases had a negative P.C.T. i.e. there was not penetration of cervical mucus by the spermatozoa, although the seminogram done before showed sperms with good progressive motility, 30% patients had a poor post-coital invasion. Only 6% cases had a satisfactory P.C.T. and still had not received upto 2 years of their married life (Graph V).

It is also seen that P.C.T. becomes poorer as the sialic acid content of the cervical mucus increases (Graph V).

GRAPH - VI. Comparison of PCT in Control and experimental groups vs. Sialic Acid concentration.

C = Control.
E - Experimental.



DISCUSSION

D I S C U S S I O N

In clinical terms, the cervical factor is assessed by an appraisal of its physical properties in relation to other criteria of ovarian function such as basal temperature charts and hormonal assays.

In my work, the study was done on 60 patients of age group 19-30 years i.e. the reproductive age group. Patients were of known L.M.P. and regular menstrual cycle of 30 days. Day 15 was taken as the day of ovulation. Samples were collected during the ovulatory mid-cycle i.e. 15 ± 2 days. Only 16 patients were such who could be studied in the pre-ovulatory, ovulatory as well as post-ovulatory phase. Regular follow up of the patients was done and the relationship of the sialic acid content of the mucus to the various physical properties of the mucus was studied. 10 patients were kept on hormonal treatment to detect the effect of oestrogens on the quality and quantity and sperm penetration of the cervical mucus.

The study of the cervical mucus and its sialic acid content has been done by a very few authors in several cycle of the same patient and for several days of the same menstrual cycle.

Carlborg, Mc. Cormick and Gemzell, C. in 1968 studied sialic acid in the cervical mucus during 9 ovulatory cycles in 5 subjects.

Daily measurements of sialic acid content and sperm penetration was done in one ovulatory cycle in 6 regularly menstruating women by Carlborg, L.; Johannson, B. and Gemzell, C. in 1969.

Mac Donals, R. and Lumley I, in 1970 made a preliminary study of 105 samples before, during and after ovulation.

Iacobelli, 1971, Carcea, N. and Angeloni, C. made a comparative analysis of the cervical secretion from pre-ovulatory, post ovulatory and pregnancy periods of 20 healthy ovulating women with regular menstrual interval of 28-30 days.

Malik, Dhar and Dhar 1974 analysed the samples obtained routinely from all cases on 8th (post-menstrual), 13th (pre-ovulatory), 17th (post-ovulatory) and 23rd (pre-menstrual) days of the cycle considering 14th day as the day of ovulation.

Kamran, S. Moghissi and Frank, N. Syner in 1976 selected 5 normal ovulatory women between the ages of 19 and 23 and studied the cervical mucus daily in mid-cycle from day 10 to 20 and every 2 days during the rest of the cycle.

SIALIC ACID AND AMOUNT OF CERVICAL MUCUS :

The amount and sialic acid concentration of the cervical mucus was determined and their relationship to each other was studied. The cervical mucus quantity was measured in terms of ml (o.1 ml or less as scanty, 0.2-0.3 ml as moderate and 0.4 ml or more as copious). I found that 10% of fertile females had 0.4 ml or more and the sialic acid was 23.60 ug/ml. 90% females had 0.2-0.3 ml i.e. moderate quantity of mucus at ovulation and the sialic acid was 25.35 ug/ml of wet mucus. Among the 50 infertile patients of the experimental group, 38% had 0.1 ml with a sialic acid of 48.68 ug/ml, 62% had 0.2-03 ml with a sialic acid of 47.39 ug/ml. No patient had 0.3 ml or more of the quantity. There was lamost no reduction in the sialic acid of ovulatory mucus in the infertile females.

The quantity was graded as copious, moderate, scanty and nil by Malik, Dhar and Dhar. They studied 140 fertile menstruating women between the ages 17-40 years from June 1972 to April 1974 and observed that 63.12% cases had a scanty, 12.28% had moderate and 22.59% had copious secretion.

The findings of my study group correlate well with the findings of Moghissi, S. and Syner, F.N., 1976 who measured the amount as mg of wet mucus and sialic acid

as mg/ml of wet mucus. They found that the amount varied from subject to subject and ranged between 16.4 - 373.7 mg in follicular phase (S.A. = 0.72 - 1.31 mg/ml), 56.3 -571.2 mg in mid-cycle (S.A. = 0.42 - 0.54 mg/ml) and 4.5 -239.3mg during the luteal phase. (S.A. = 0.65 - 4.21 mg/ml). The ovulatory fall of the sialic acid as seen in the above study was not found in my study in the 16 infertile females instead they had almost the same high content of the sialic acid with only a minor increase in the quantity at mid-cycle from 0.1ml to 0.15 - 0.2 ml at ovulation. This absence of the mid-cycle fal of sialic acid could be reponsible for the infertility.

SIALIC ACID AND VISCOSITY OF MUCUS :

An increased sialic acid increases the viscosity of the mucus which in turn hampers sperm penetration. This association has been seen in my study. Among the 10 control patients, 70% had a low viscosity and a low sialic acid of 23.81 ug/ml and 30% had a moderate viscosity and a little higher sialic acid of 30.0 ug/ml.

Among the 50 infertile patients 5% had a high viscosity with a high sialic acid (50 ug/ml) and 42% had a moderate viscosity with sialic acid 47.03 ug/ml and 48% had a low viscosity with a relatively low sialic acid 46.82 ug/ml but it was almost the double of the fertile group.

A direct association between viscosity (measured by consistometer and sperm penetration (measured by haemocytometer) was demonstrated by Viergiver and Pommerenke , 1946, the very thin mucus at mid-cycle corresponded exactly to the maximum rate of sperm penetration and a lowering of sialic acid.

The change of viscosity is due to the functional role played by the sialic acid. This was proved by Gibbons and Kattner in 1967.

The relationship of viscosity to sperm penetration was further confirmed by Mac Donald and Lumley in 1970 on 105 patients who found that the thicker samples of mucus which were impenetrable to sperms showed a reverse pattern when saline was added to the sample and viscosity reduced. The sperm penetration increases with a reduction in viscosity which in turn is due to a reduction in the sialic acid.

SIALIC ACID AND SPINABILITY OF CERVICAL MUCUS

80% of the fertile patients of my study had a high spinbarkeit i.e. 7 10 cms and a simultaneous low sialic acid i.e. 22.47 ug/ml and 20% had a spinability of 7-10 cms with a sialic acid a little higher i.e. 31.0 ug/ml but among the infertile patients this pattern was absent.

6% patients with a poor spinability had a sialic acid of 45.43 ug/ml and 60% patients with a slightly better

spinability had a still higher sialic acid equal to 48.23 ug/ml. Only 2% patients were in accord with the findings of Carlborg et al, 1968, Carlborg, Mc Cormick Gemzell,1969 and Moghissi and Marks, 1971.

SIALIC ACID AND FERN PATTERN OF CERVICAL MUCUS

The degree of ferning in this study was graded as 1+ to 4+. Extent of ferning has a direct relationship to viscosity and spinnbarkeit and sperm receptivity. The sialic acid were determined in relation to the above properties and it was found that 38% of infertile patients had a scanty mucus and a high sialic acid i.e. 48.68 ug/ml. 6% had a poor spinnbarkeit and a high sialic acid i.e. 45.43 ug/ml and minimum ferning with high sialic acid i.e. 49.15 ug/ml. This relationship of sialic acid to fern phenomenon has only been stated by Carlborg, Mc Cormick and Gemzell, 1968 and Moghissi and Marks in 1971, but a relationship between fern and spinnbarkeit was studied by Jain et al, 1973 and Malik, Dhar and Dhar, 1974. They found that 35.54% had a positive fern and 35.68% a positive spinnbarkeit, that is the two properties go hand in hand and are maximal at the time of ovulation.

SIALIC ACID AND pH OF CERVICAL MUCUS

No relationship between the sialic acid and pH was found in the present work. 70% of fertile patients with

a pH 7.0 had a low sialic acid i.e. 25.04 ug/ml and 76% of infertile females also had a pH 7.0 but a higher sialic acid of 55.23 ug/ml. 24% patients with an acidic pH of mucus also had a high sialic acid (51.03 ug/ml). Thus sialic acid is not related to the pH of the cervical mucus and vice versa and no relationship had been mentioned in literature too. Patients with a pH of 7.0 or less were given alkaline pre-coital douches.

SIALIC ACID CONTENT OF CERVICAL MUCUS

Sialic acid was estimated by the method of Aminoff given in 1959 for the quantitative estimation of N-acetyl neuraminic acid in sialomucoids. NANA from E.Coli, Sigma 98 f crystalline, Anhydrous Mol. wt 3093 (M/S Sigma Chemicals, U.S.A.) was used as standard, obtained from the Biochemistry department of the All India Institute of Medical Sciences, New Delhi.

The mean sialic acid was found to be 25.67 ug/ml of wet mucus at ovulation in fertile females and highly significant rise to 47.32 ug/ml of wet mucus was found among the infertile females.

The first study on the content of sialic acid in the cervical mucus was done by Gibbons, R.A. in 1959. Sialic acid was determined by him also according to the method of

Aminoff after hydrolysis of the sample in 0.1 N sulphuric acid for 30 min. N-acetyl neuraminic acid (Sigma Chemicals Co., St. Louis, Mo.) was used as standard. The sialic acid concentration was found to be 30 ± 2.8 ug/ml in pre-ovulatory to 46 ± 4 and 49 ± 5.5 ug/mg of dry mucus in post-ovulatory and pregnancy samples.

Carlborg, L., Johansson, D.B. and Gemzell, C. in 1968 determined the sialic acid to an average of 21 ug/ml of dry mucus (range 12-31 ug/ml) at mid-cycle and it increased in the luteal, that is the post-ovulatory phase. The method used was the Direct Ehrlich method (Werner & Odin, 1952).

Carlborg, Mc Cormick and Gemzell again in 1969 determined sialic acid to be at its lowest value i.e. 20-25 ug/mg of dry mucus on day 16, followed by a rapid increase.

Jacobelli et al, 1971, found the average sialic acid to 30.0 ug/mg of dry mucus at ovulation. Kamran, S., Moghissi, and Frank, T. Syner, 1976 analysed the ovulatory samples of mucus and found it to range between 0.42 to 0.54 mg/ml of wet mucus. The method used was that of Warren, L. (The Thiobarbituric acid assay of sialic acid).

Estimation of sialic acid was also done by Khuteta et al, 1985 by the principle of Tryptophane perchloric acid reaction by Siebert et al (1984) and the average detected was 28.4 ± 3.55 ug/mg at ovulatory period in fertile females and 46.6±10.1 ug/mg of dry mucus in infertile females.

SIALIC ACID AND POST-COITAL TEST

Mid-cycle fall of sialic acid is associated with an increased sperm penetration. This observation was noted in my study. A study on 10 fertile females in this work showed a low mid-cycle sialic acid amounting to 25.67 ug/ml and a satisfactory PCT. 64% of the infertile females had a sialic acid of 52.43 ug/ml with a negative PCT, 30% with sialic acid equal to 40.30 ug/ml had an unsatisfactory PCT and 6% with a low sialic acid i.e. 27.66 ug/ml had a satisfactory PCT.

Carlborg et al in 1968 studied the cyclic changes of sialic acid. He found that in 19 out of 25 cycles, cyclic changes in the sialic acid were found together with a positive sperm receptivity and the patients if treated with combination therapy, no cyclic changes in sialic and concentration and no sperm receptivity was seen.

In a second experiment in 1969, a similar observation was noted, that is all the 6 patients with a low sialic acid i.e. 12 - 31 ug/ml at ovulation, showed a peak sperm penetration.

CONCLUSION

C O N C L U S I O N S

Following conclusions could be made from our study :

1. The average sialic acid in cervical mucus estimated by the method of Aminoff (1959) is 25.67 ug/ml of wet mucus at ovulation in fertile females and a higher value to almost the double i.e. 47.32 ug/ml in the infertile females.
2. All the patients of the control i.e. fertile group had a low sialic acid i.e. 23.60 ug/ml in 10% and 25.35 ug/ml of wet mucus in 90% and a moderate or abundant quantity signifying that the lower the sialic acid, the more the quantity but this was not seen in the infertile group where irrespective of the quantity, the sialic acid was high.
3. An increased sialic acid increases the viscosity of the cervical mucus which in turn hampers sperm penetration.
4. The spinability of the cervical mucus increases with a fall in sialic acid content of the cervical mucus.
5. The degree of ferning has a direct relationship to viscosity, spinnbarkeit and sperm receptivity which in turn is affected by the sialic acid of the mucus.
6. The ferning property and spinability go hand in hand and are maximum at ovulation when sialic acid is at its minimum.

7. Sialic acid has no relationship to the pH of the cervical mucus nor is the pH affected by the sialic acid content.

8. Cyclic changes in the sialic acid were found together with a positive sperm receptivity and a minimal sialic acid at ovulation shows a peak sperm penetration.

9. Treatment was implicated on 10 patients of my experimental group in the form of :-

(1) Hormones :- Ethinyl oestradiol (Lynorel) 0.01 mg daily from 5th to 15th day of the menstrual cycle for 3 cycles or 0.05 mg daily from 5th to 9th of cycle for 3 cycles was given to the patients showing a negative or poor post coital test.

Response :- A marked improvement in the quantity and quality of the cervical mucus was seen. Previously a poor PCT was found to be satisfactory after 4-5 months of treatment with hormones and this was in 5 out of the 10 cases chosen.

(2) Pre-coital alkaline douches : This was indicated in patients having a pH of 7.0 or less. A 3.75% of soda bicarb solution was prepared and patients were advised to douche their vagina half an hour before coitus in the pre-ovulatory and ovulatory phase for about 5 months.

Response :- PCT which initially had a fair number of immotile sperms showed an improvement in the motility and as such better post-coital invasion.

One patients with an infertility of 4 years conceived 5 months after the treatment when oestrogens, antibiotics and alkaline douches were advised together. Four patients did not turn up and their response could not be known.

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S U M M A R Y

Penetration of cervical mucus by a large number of sperms is essential to fertility. This necessitates both the proper deposition of normal semen and a salutary state of the cervical canal. The association of a normal semen with a poor post-coital invasion, is a cause of infertility in 5-8% of barren women, where other investigations of infertility do not show any abnormality as a cause.

Cervix is a factor in sterility because of the pathological changes in the physical characteristics and the chemistry of its mucus. Even clear, elastic, ovulatory cervical mucus may exhibit hostility.

The sialic acid (a glycoproteins) an improtant constituent of the cervical mucus responsible for the rigidity and coherence of the mucin molecule has been found applicable to many more conditions then the other tests of the cervical mucus.

The present attempt has been made on 60 patients and the clinical assessment of this cervical factor has been done in relation to its place in the investigation of an infertile female.

60 patients were chosen from the outdoor patients department of the M.L.B. Medical College Hospital from the

Department of Obstetrics and Gynaecology during the period August '88 to June '89. The women were clinically diagnosed as being infertile and with husband having normal fertility status. Patients were of known L.M.P. and regular menstrual cycles and not taking any contracystive measure. A detailed history was taken and examination was done consisting of general, physical, systemic, P/S and P/V examinations.

10 patients of these with proved fertility were taken as controls.

Samples of mucus were collected in the ovulatory mid-cycle i.e. 15 $\pm$ 2 days and the sample was collected from the endocervical canal with the help of an insulin syringe by repeated aspirations. Specimen was stored at -18^{o}C unless the sialic acid analysis could be made.

Evaluation of the physical properties such as quantity, viscosity, spinnbarkeit, pH ferning and PCT was done along with the quantitative estimation of sialic acid (N-acetyl neuraminic acid) by the method of Aminoff 1959). N-acetyl neuraminic acid from E. coli, Sigma 98f anhydrous Mol. weight 3093 (M/s Sigma Chemicals, U.S.A.), obtained from the AIIMS, New Delhi was used as standard.

The study of cervical mucus and its sialic acid content has been done by a very few authors in several cycles of the same patient and for several days of the same

menstrual cycle. In the present work, the mucus was studied mainly in the ovulatory phase of a 30 days cycle. Only 16 out of 60 patients could be studied in all the three phases.

Relationship of sialic acid to the various physical properties of the mucus and to the sperm penetration was determined. Further a correlation between the sialic acid and sperm penetration was determined. Sperm penetration was found to be higher with a lowering of sialic acid due to a lowering of viscosity of the mucus. This was also confirmed by Carlborg et al in 1968-69.

Sialic acid fall at ovulation was found to be associated with a decreased viscosity (Viergiver and Pommerenke, 1946), increased spinability (Carlborg et al, 1968), increased ferning (Carlborg, Mc Cormick and Gen Zell, 1968 and Moghissi and Marks, 1971) and increased amount (Syner, F.N., 1976). A similar relationship was seen in this study. No significant change in pH was detected with a change in sialic acid.

CONCLUSION

1. The cyclic alterations of the cervical mucus are responsible for the periodic receptivity or hostility of the hydrogel to sperm invasion.
2. The concentration of sialic acid decreases during the proliferative phase to reach a minimum at ovulation and this fall in the sialic acid is associated

with a positive sperm receptivity while in patients with no change in sialic acid no sperm receptivity was seen.

3. The average sialic acid as determined by the method of Aminoff, 1959 was 25.67 ug/ml. of wet mucus at ovulation in fertile females and almost double i.e. 47.32 ug/ml in the infertile females.

4. Patients treated with oestrogens (Ethinyl oestradiol) 0.01 mg daily from 5th to 15th day of menstrual cycle or 0.05 mg from 5th to 9th day of cycle showed a significant improvement in the quantity and quality and the post-coital invasion of the mucus by sperms.
